HYALURONIC ACID AUTO-CROSSLINKED POLYMER (ACP®): REACTION AND PARTICLE SIZE MONITORING, POLYMER CHARACTERIZATION AND HYALURONIDASE STABILITY

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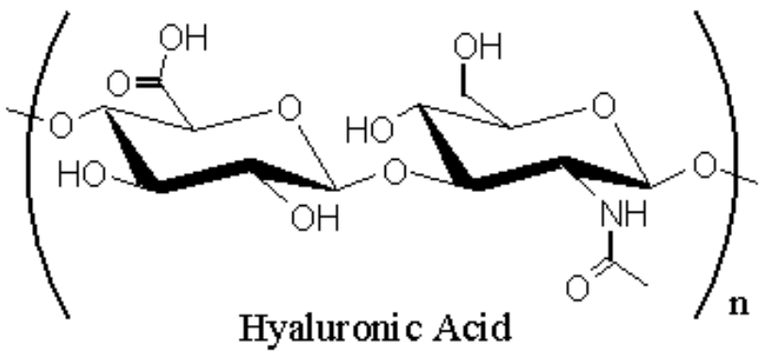
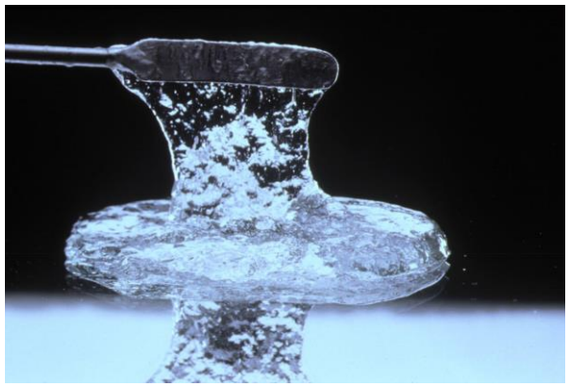
Hyaluronic Acid (HA)…

HA is a linear anionic non-sulfated glycosaminoglycan widely distributed throughout the body



Hyaluronic Acid



HA finds several application due to its:

- High hydro-solubility
- Excellent biocompatibility
- Great lubricating properties

…but…

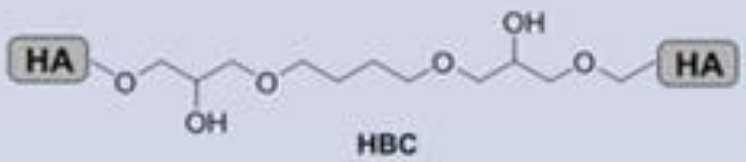
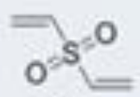
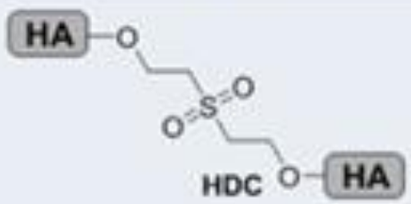
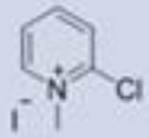
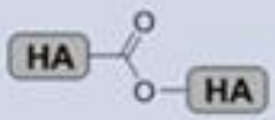
…unmodified HA hydrogels are mechanically too weak to provide sufficient support when used in the body(1)

…unmodified HA is readily degraded in vivo by hyaluronidases and free radicals(2)

(1) J. Biomater. Sci. Polymer Edn 17 (2006) 419-433
(2) Biotechnology Adv. 25 (2007) 537-557

…and Crosslinked HA

- **Chemical cross-linking** is an effective method to enhance the bio-stability of the polymer structure of HA.

Crosslinking agent	Crosslinked HA derivative
 BDDE	 HBC
 DVS	 HDC
 CMPI	 ACP

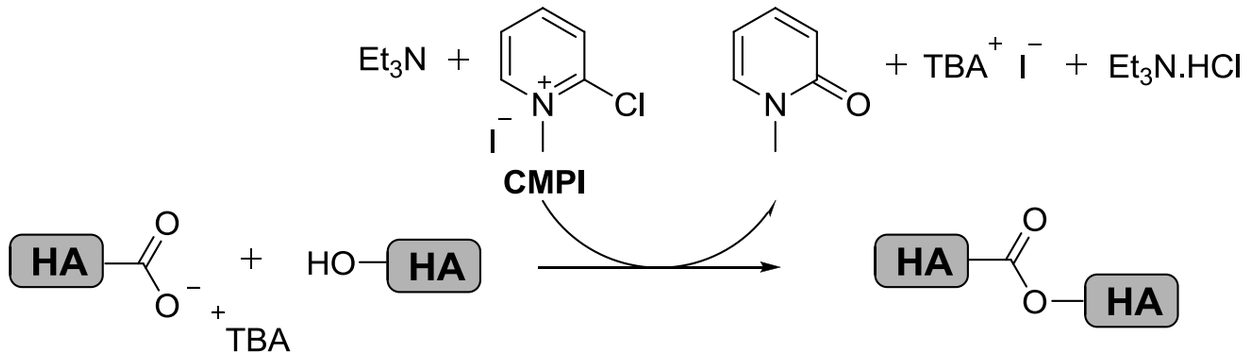
Bi-functional crosslinking agents (DVS[3], BDDE[4], etc…)

Zero-length crosslinking agent: 2-Chloro-1-Methylpyridinium Iodide (CMPI)

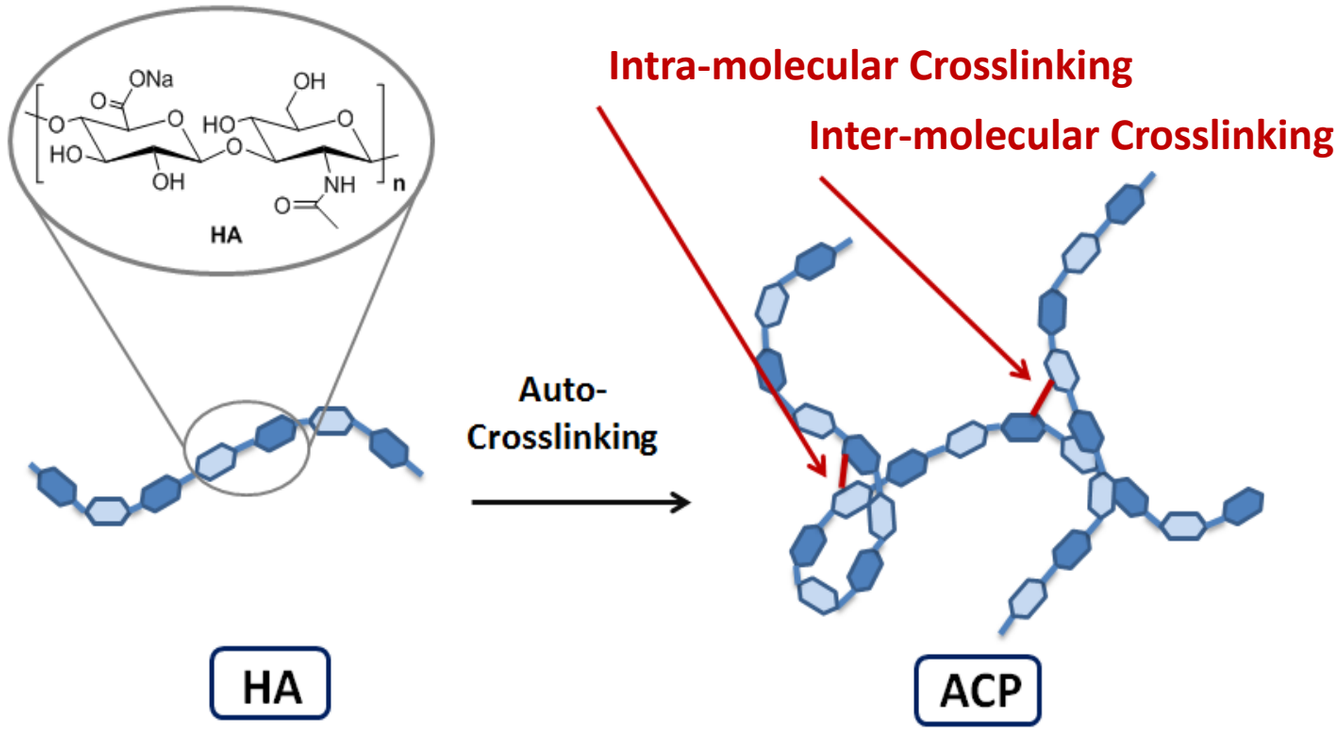
- **Bi-functional crosslinked HA** shows some risks of toxicity, but high biostability.
- **Zero-length crosslinked HA** shows no risks of toxicity, higher biocompatibility and water solubility, but exhibits lower biostability[1].

(3) J. Appl. Polym. Sci. 5 (2007) 3183-3191
(4) Carbohydr. Polym. 88 (2012) 428-434

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®(5)) - Synthesis



- Reaction performed in N-Methyl-2-pyrrolidone (NMP) starting from HA TBA salt
- Activation of carboxyls of HA by CMPI and nucleophilic attack by hydroxyls of HA



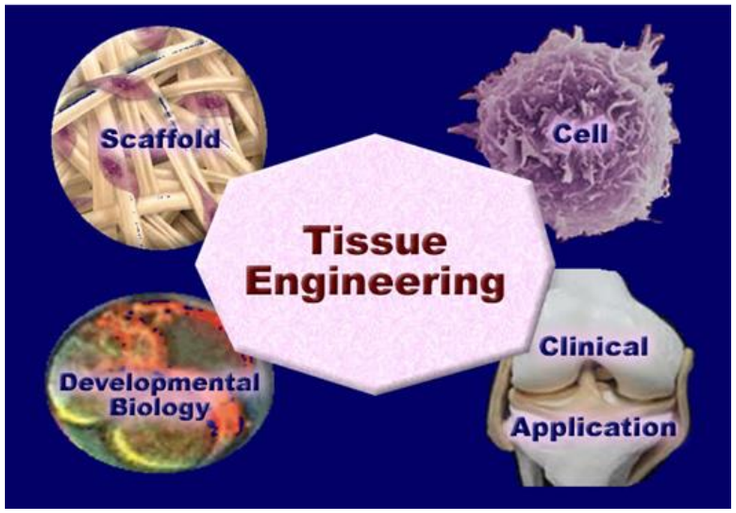
- Rheological properties of the product are strongly influenced by the crosslinking density (ratio intra-/inter-molecular crosslinking)(6)

(5) ACP® patent: EP0341745 (1989)
(6) Biomaterials 23 (2002) 1161-1167

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®)

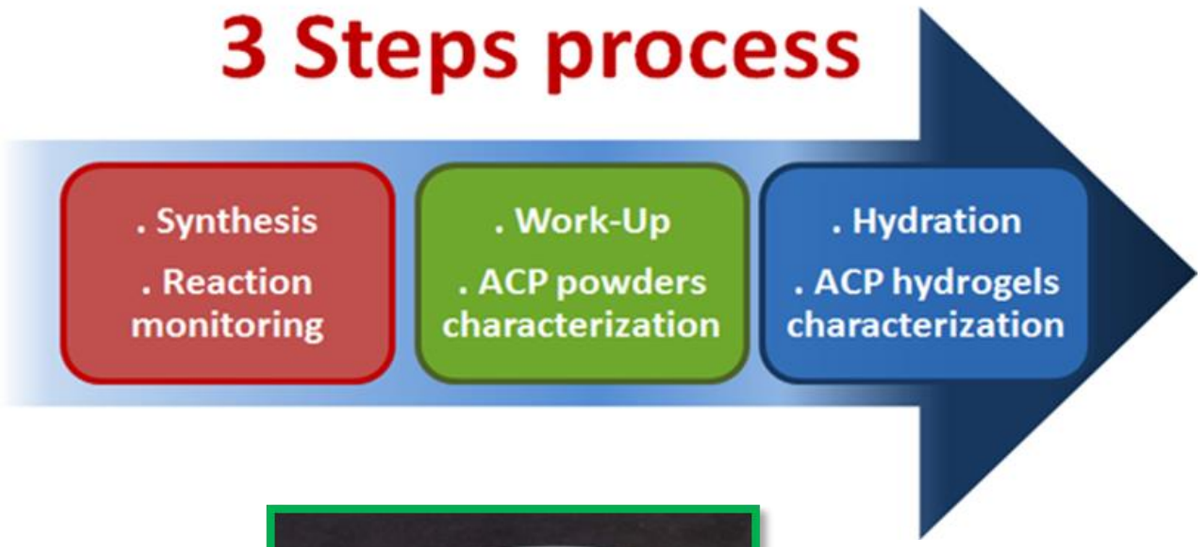
ACP® biomedical applications:

- Prevention of adhesion in post surgical applications[7]
- Dermal Fillers[8]
- Tissue Engineering[9]



(7) Biomaterials 26 (2005) 5368-5374
(8) Plast. Reconstr. Surg. 118 (2006) 341-346
(9) Carbohydr. Poly. 92 (2013) 1262-1279

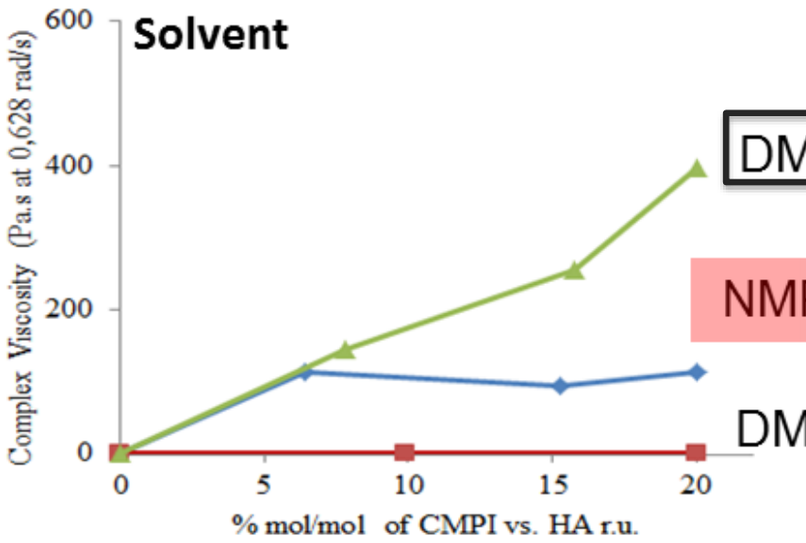
Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) - Process



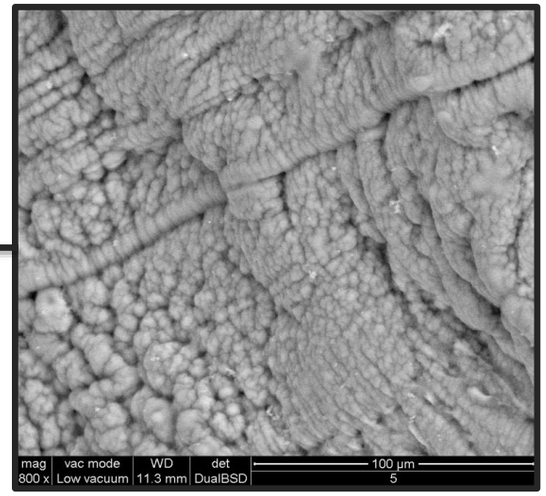
- Unchanged industrial process since 1989
- Work-up includes 2 precipitations, washings and vacuum drying
- About 3 weeks from Synthesis to perform work-up and obtain dry ACP powder
- Analysis performed only once dry powder is obtained
- Controlled and standardized rheological properties of the product are fundamental because specific ACP® hydrogel performances are required

ACP® patent EP0341745 (1989)

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) – Solv+Temp choice

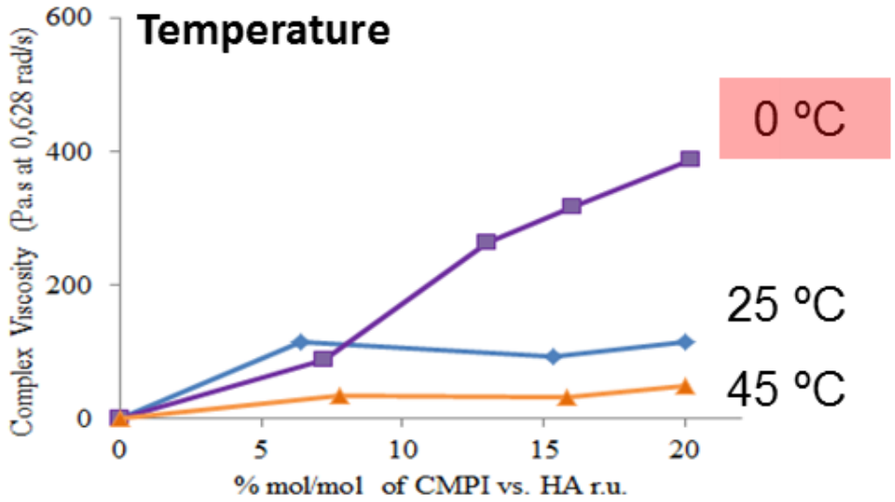


DMF



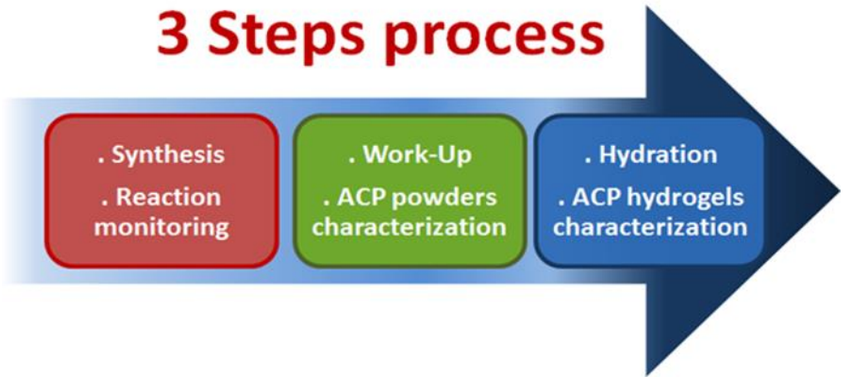
NMP

DMSO

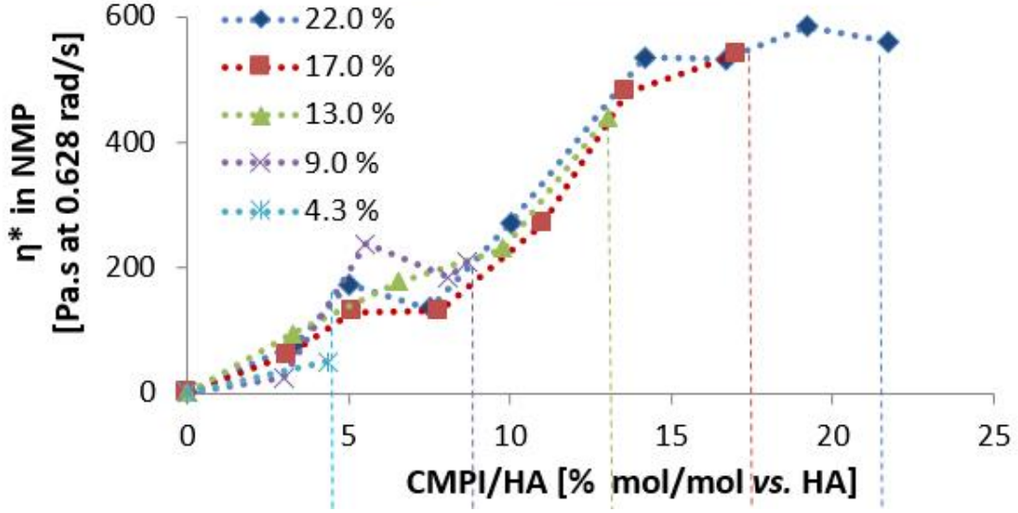


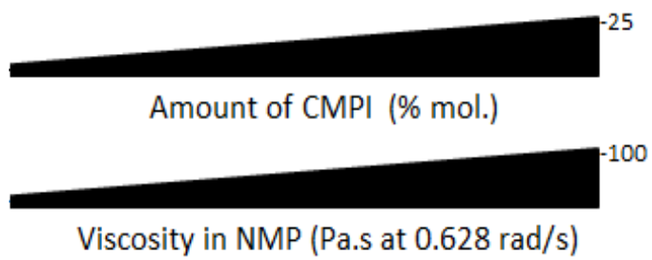
- Better rheological properties if synthesis is carried on in DMF but dry powder porosity is preferable when synthesis is performed in NMP
- Wide range of rheological performances when synthesis in NMP is performed at 0°C

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) - Monitoring



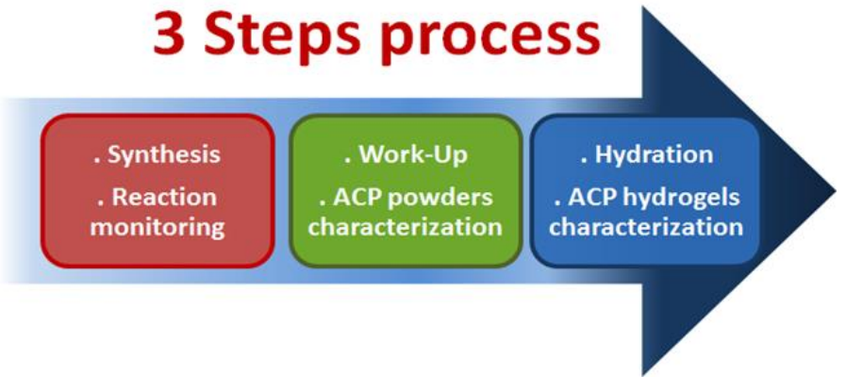
- Reaction Monitoring performed through complex viscosity measurement

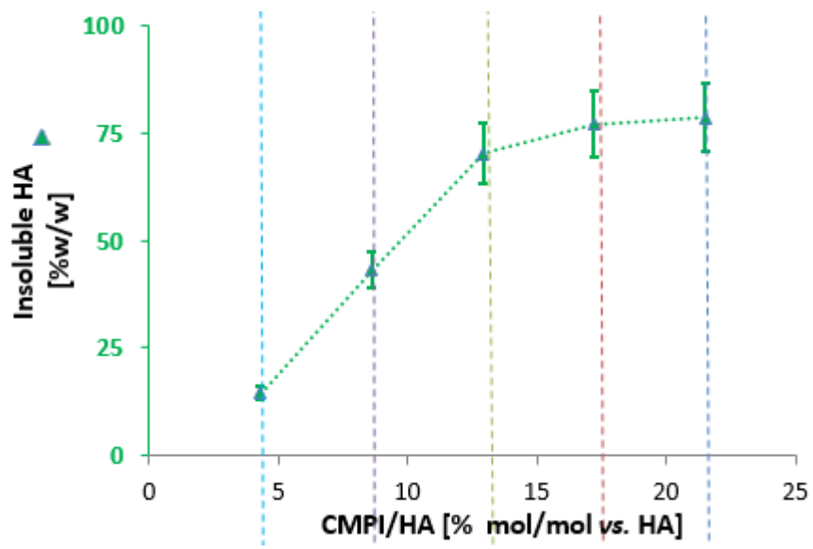


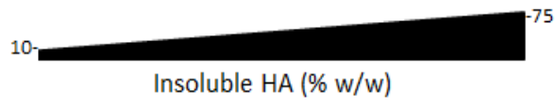


- Batches reproducibility at different amount of added CMPI
- Linear correlation between added CMPI (% mol) and Complex viscosity

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) – Work Up

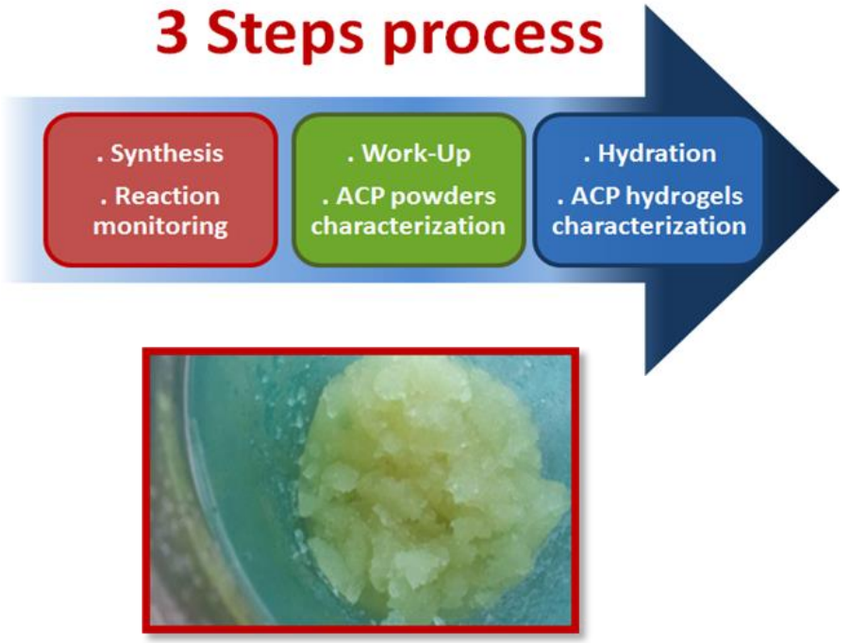


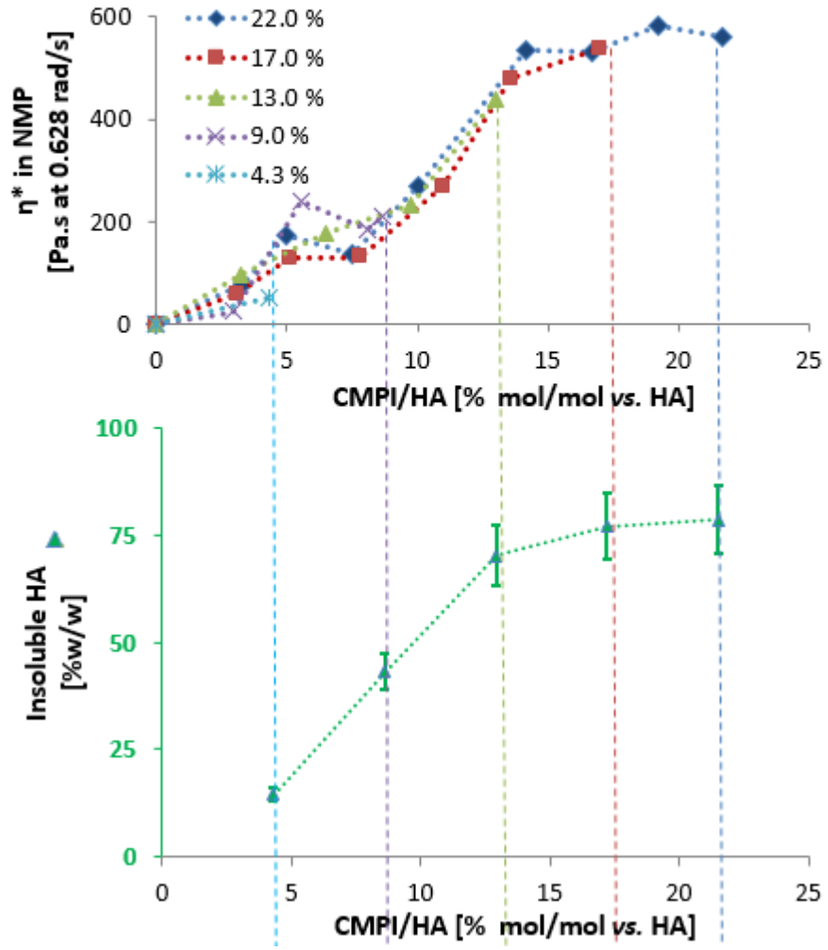


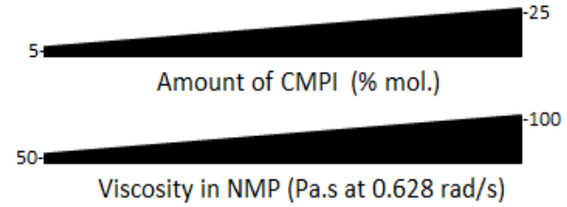


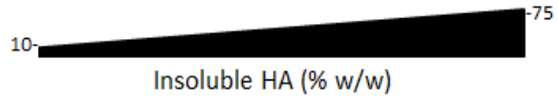
- Soluble HA fraction measured by means of GPC (Insoluble HA = Total HA – Soluble HA)

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) – Work Up



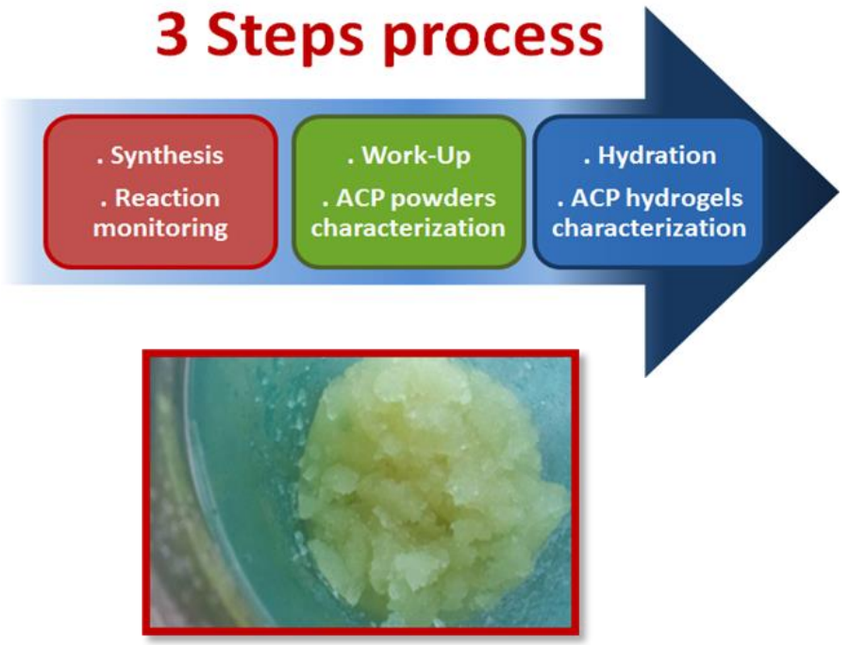


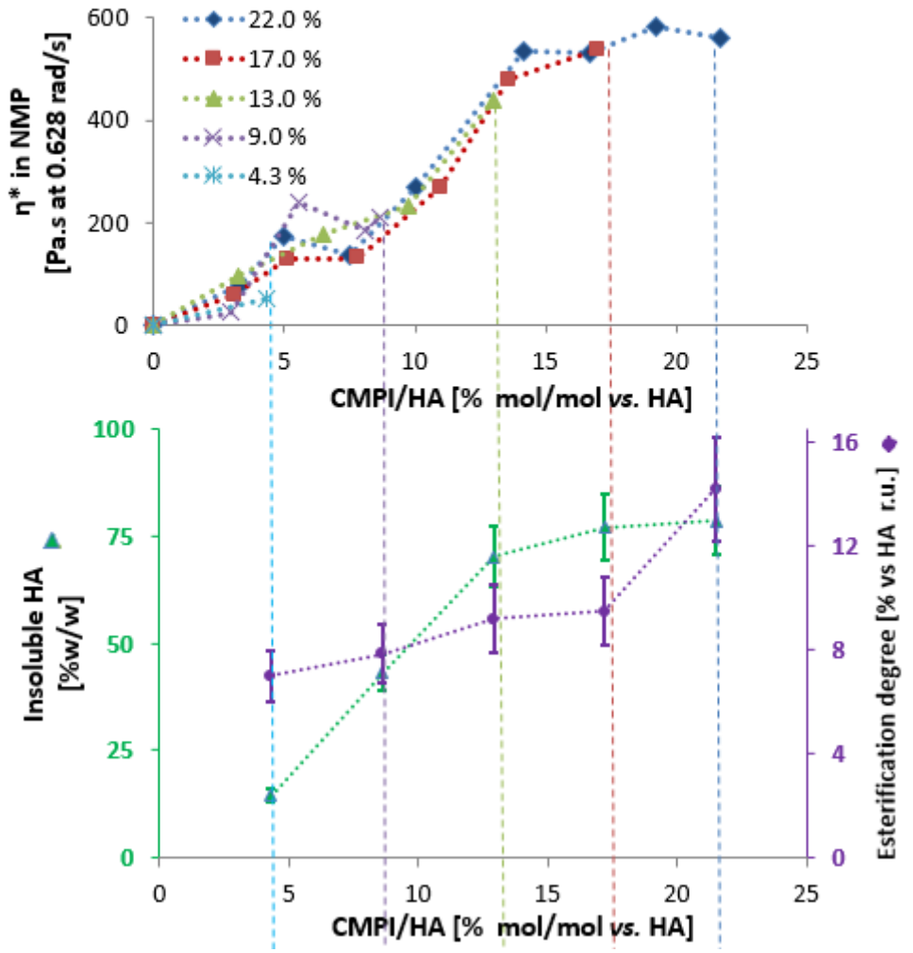


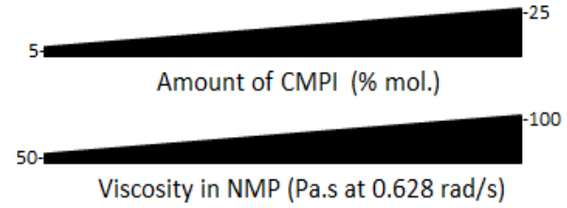


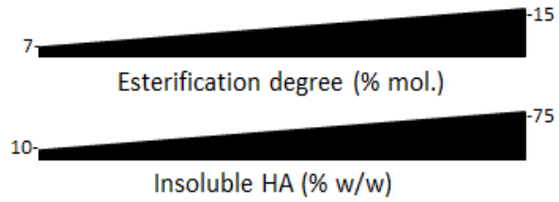
- Soluble HA fraction measured by means of GPC (Insoluble HA = Total HA – Soluble HA)
- Linear correlation with reaction parameters (CMPI amount and complex viscosity)

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) – Work Up









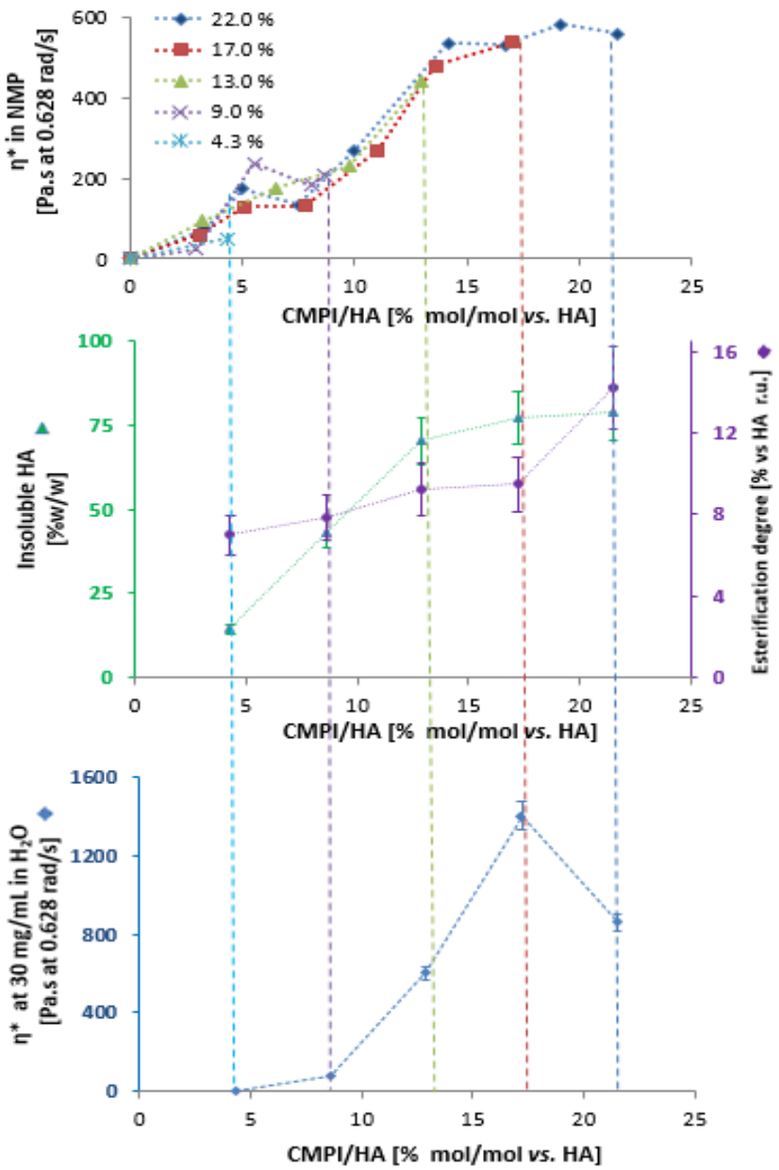
- Esterification degree determined by colorimetric quantification of ferric hydroxamate complexes(10)
- Linear correlation with other tested parameters

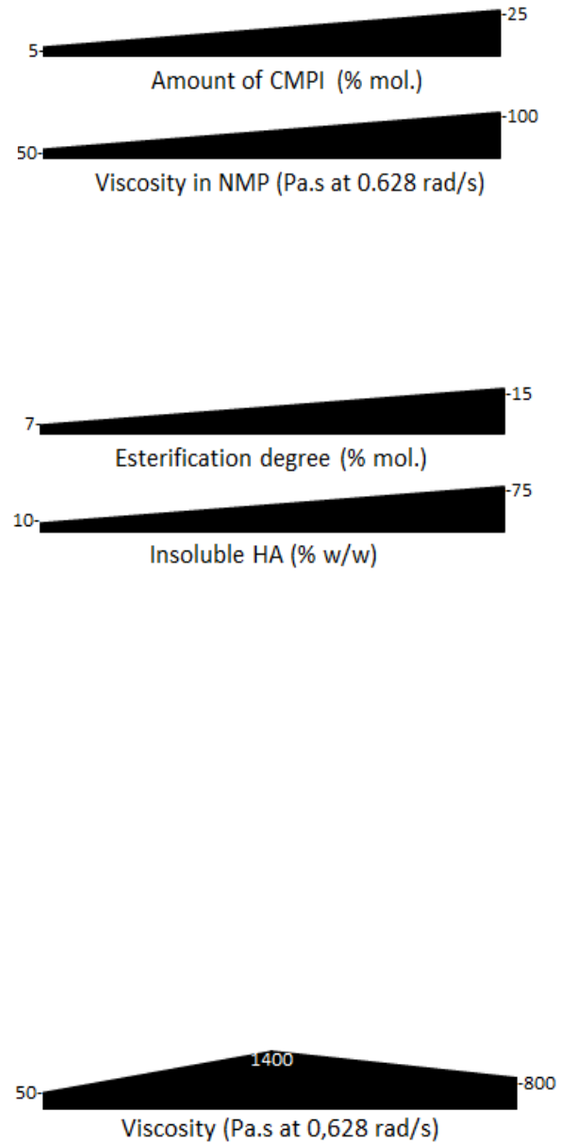
(10) Anal. Chem. 29 (1957) 819

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) - Hydration

3 Steps process

- . Synthesis
  . Reaction monitoring
- . Work-Up
  . ACP powders characterization
- . Hydration
  . ACP hydrogels characterization





- Final product formulated in isotonic saline at 30 mg/mL
- Unexpected drop in complex viscosity of the final product

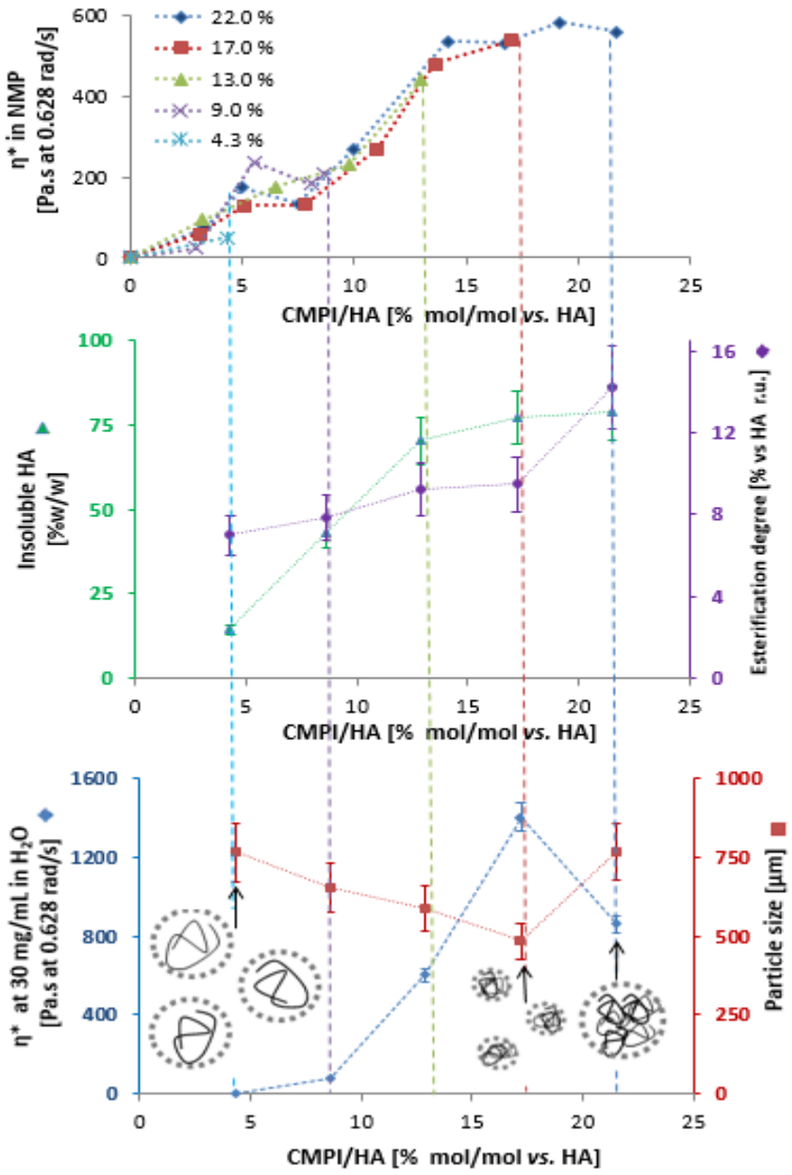
Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) - Hydration

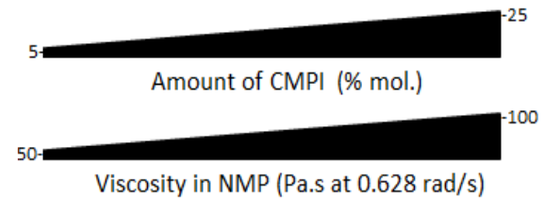
3 Steps process

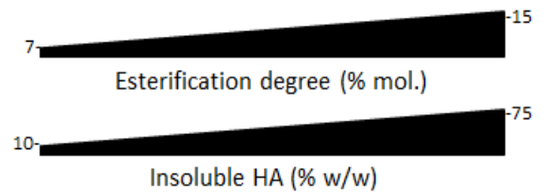
. Synthesis
. Reaction monitoring

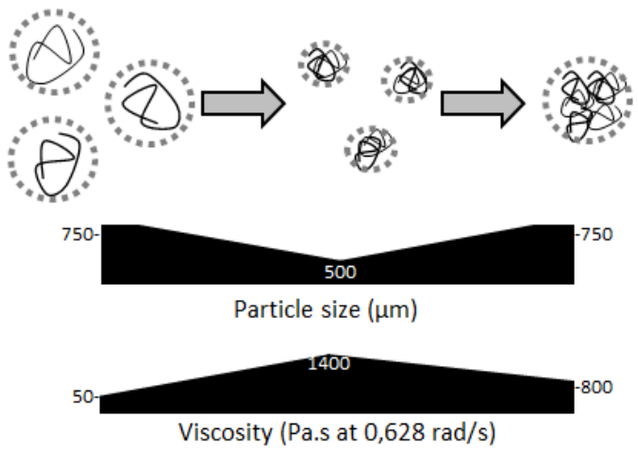
. Work-Up
. ACP powders characterization

. Hydration
. ACP hydrogels characterization





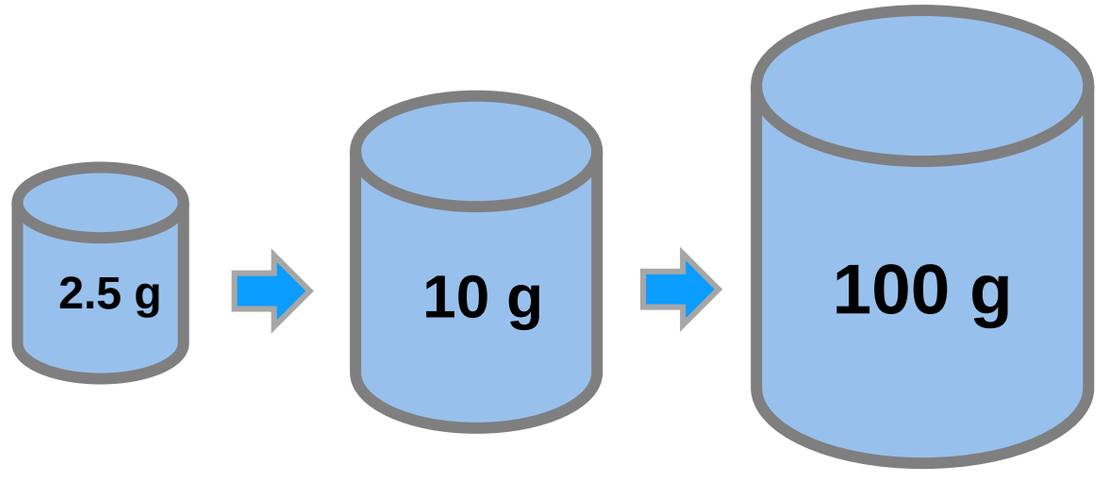


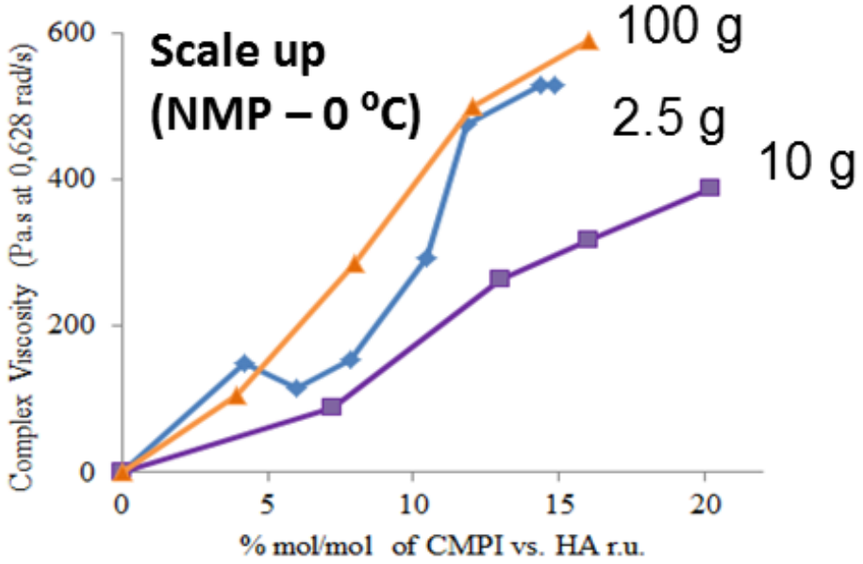


Two steps mechanisms proposed: 1. up to 16% mol/mol CMPI, **cluster contraction** is promoted
2. over 16% mol/mol CMPI, **external crosslinking** of HA clusters

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) – Process scale-up

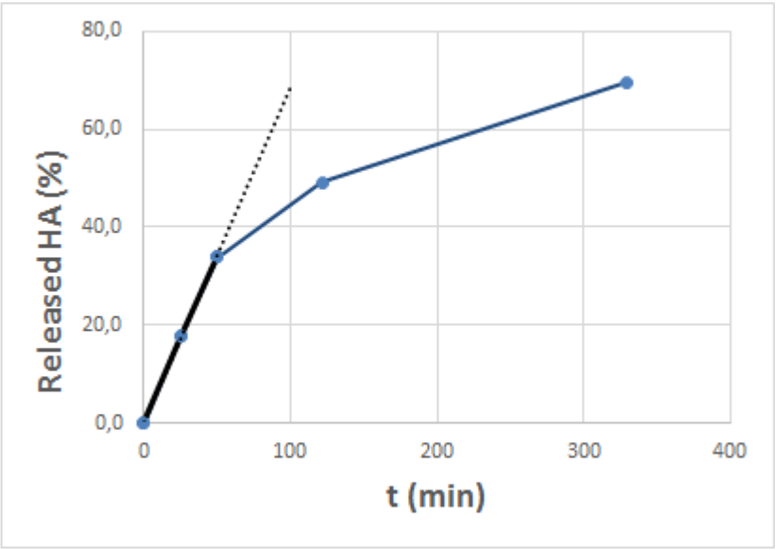
- Scale-up tested at three different batch sizes





- Good reproducibility, some difference probably due to the geometry of reactors

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) – HYase stability



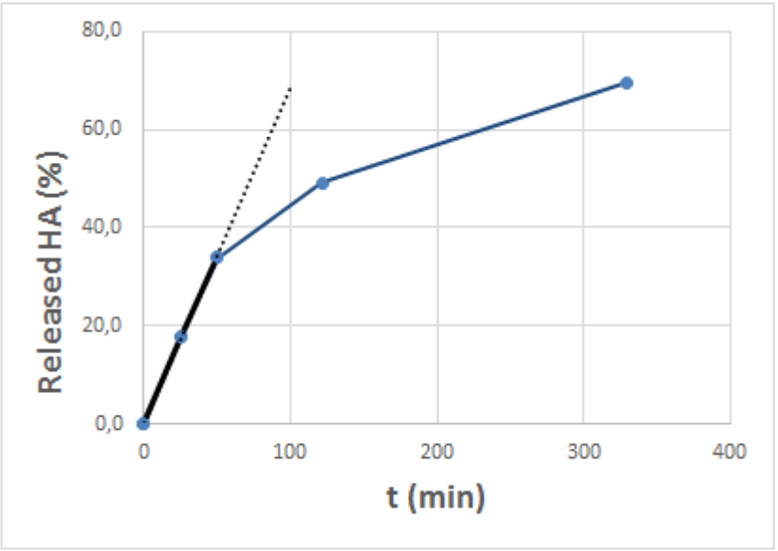
Experimental conditions[11]:

- Zero-length (ACP) *vs* bi-functional crosslinked HAs (HBC, HDC)
- Several synthesis at Increasing elastic modulus (G')
- Same concentration (0.5 mg/mL in PBS 6.4)
- Bovine Testicular Hyaluronidase digestion (10 U/mL at 37°C)
- Released HA measured by GPC (0, 0.5, 2.25, 5.5, 19 hours)
- $V_{initial}$ is the initial rate constant of the first order kinetic (released HA *vs* time)

Crosslinking agent	Crosslinked HA derivative
BDDE	HBC
DVS	HDC
CMPI	ACP

(11) Carboh. Res. 433 (2016) 47-53

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®) – HYase stability



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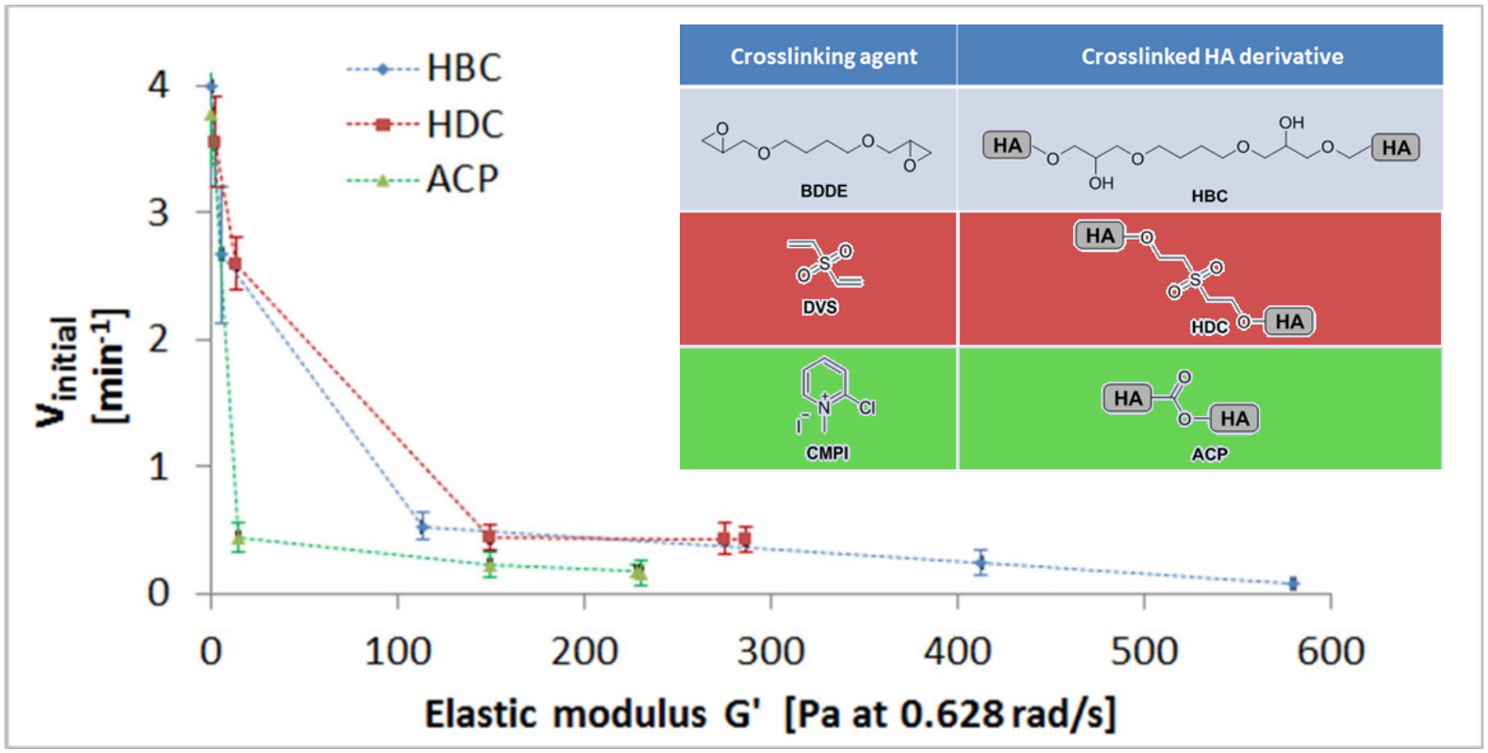

- At low elastic modulus values the ACP resistance to BTH hydrolysis is higher compared to HBC and HDC.
- ACP conformation is likely limiting substrate accessibility to Hyaluronidase.

(11) Carboh. Res. 433 (2016) 47-53

Hyaluronic Acid Auto-Crosslinked Polymer (ACP®)

CONCLUSIONS

- Solvent and temperature effect on crosslinking reaction have been optimized.
- Rheological analysis provided a valuable tool to properly monitor the physicochemical properties of ACP intermediates.
- ACP powders and hydrogels have been characterized to establish the correlations between CMPI concentration and esterification degree, water-insoluble fraction, complex viscosity and particle size.
- Process reproducibility and robustness have been achieved as a result of a scale-up phase.
- The enzymatic hyaluronidase assay gave an unexpected result: the rate constant of ACP cleavage, at low elastic modulus, is lower than that of bi-functional crosslinked HAs

THANK YOU!

…need more info?

Carbohydrate Research 433 (2016) 47–53

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Hyaluronic acid auto-crosslinked polymer (ACP): Reaction monitoring, process investigation and hyaluronidase stability

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